them to secure the cooperation of persons interested in the enforcement of existing laws, for the purpose of securing the enactment of the legislation necessary to provide for a separate classification as dealers in alcohol and narcotic drugs, with the requirement that dealers in this class keep a record sufficient to show the amount of alcohol or alcohol-containing materials purchased, and thus afford to officials in prohibition or local-option territory an opportunity to actually enforce this type of restrictive legislation in a way hitherto impossible.

ROCKY MOUNTAIN SPOTTED FEVER.

A REPORT OF LABORATORY INVESTIGATIONS OF THE VIRUS.

By L. D. FRICKS, Surgeon, United States Public Health Service.

During 14 years of investigation by different workers the following facts bearing upon the nature of the virus of Rocky Mountain spotted fever have been determined:

Man, rhesus monkeys, and at least six varieties of small wild rodents found in the Rocky Mountain region are susceptible to infection, while the larger domestic animals are generally immune. Of the laboratory animals, guinea pigs and white rats (*Mus norvegicus albinus*) are highly susceptible, while white mice (*Mus musculus albinus*) are apparently immune.

The virus is transmitted to susceptible animals, including man, by the bite of infective wood ticks (*Dermacentor*), recovery being followed by complete immunity. No other biting arachnid or other insect has been found capable of transmitting the virus. The transmission is not mechanical, since a tick once infected remains so, the virus multiplies in the tick and the female tick transmits the virus to her progeny. The virus may be propagated indefinitely in guinea pigs without loss of virulence by weekly blood inoculations, but dies within a few days outside the animal body. It will not pass through an ordinary Berkefeld filter under moderate pressure, and many attempts to cultivate it aërobically in the usual laboratory media have failed.

Wilson and Chowning, in 1902, described a piroplasm in the red blood cells of Rocky Mountain spotted fever cases seen in fresh blood smears both stained and unstained, but subsequent workers have failed to confirm their findings.

Ricketts reported diplococcoid bodies occasionally seen in fresh blood smears stained with Giemsa stain and many small bacilli found in infected tick eggs. He appears to have considered these as different forms of a specific microorganism, but afterwards found similar bacilli in noninfected tick eggs. Ricketts reported the agglutination of this bacillus found in tick eggs by immune guinea pig serum in dilutions of 1 to 320, but was unable to cultivate the organism.

Recent Investigations of the Virus.

In connection with the field campaign conducted by the Public Health Service for the purpose of ascertaining the measures best adapted to the eradication of Rocky Mountain spotted fever from a community, and for determining the present areas of infection in the Rocky Mountain region, laboratory investigations of the virus have been carried on both in the field laboratory at Victor, Mont., and at the Hygienic Laboratory, Washington. It is believed that the findings are of sufficient interest to warrant a preliminary report thereon at the present time.

All attempts made to cultivate the virus on many different media aërobically have failed, despite the fact that the virus circulates freely in the blood stream, 0.1 c. c. of blood frequently being sufficient to infect a guinea pig.

Attempts were made two years ago to grow the virus anaërobically by mixing infected guinea pig blood with freshly melted and properly cooled glucose agar and glucose ascitic agar, in different dilutions, with and without the addition of normal guinea pig kidney. No uniform results were obtained; occasionally, however, anaërobic diphtheroid bacilli were encountered, but inasmuch as they were all found nonpathogenic for guinea pigs they were abandoned.

Following the announcement of the discovery of the "*Bacillus typhi exanthematici*" by Plotz, and because of the close clinical resemblance between Rocky Mountain spotted fever and typhus fever, the different anaërobic bacilli, referred to above, which have been encountered since in cultures, have been studied more carefully.

Ten strains of these anaërobic bacilli have been isolated; some from dilute guinea pig serum plus normal guinea pig kidney planted with infected blood, some from glucose ascitic agar plus normal guinea pig kidney planted with infected blood, and one from freshly boiled 1 per cent glucose broth in fermentation tube in which 5 c. c. of infected guinea pig blood had been planted.

These bacilli have not been found with anything approaching the frequency with which Plotz was able to recover "*Bacillus typhi exanthematici*" from typhus cases; but in the writer's routine work only from 5 to 10 drops of infected blood were used for planting, that amount being well above the minimum infective dose for guinea pigs. Plotz, on the contrary, regularly used 2 c. c. or more of typhus blood.

All of the 10 strains referred to are strict anaërobes, growing equally well in deep stabs on freshly melted glucose agar and ordinary agar, and in fresh glucose broth in fermentation tubes.

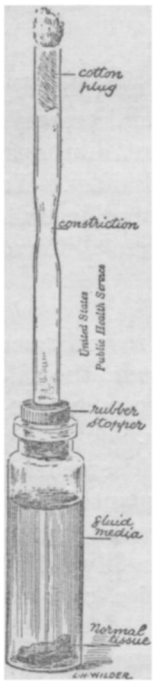
These organisms are nonpathogenic to guinea pigs, are not agglutinated by immune guinea pig serum, and do not show complement fixation with immune serum when used as antigen. The macro-

scopic method of agglutination was easily employed with cultures grown on fresh glucose broth in fermentation tubes. (Microscopically an apparent clumping of the bacilli is nearly always seen.)

These organisms, recovered from spotted fever guinea pigs, resemble very closely morphologically and culturally the two strains recovered from typhus guinea pigs by Hasseltine and Neill at the Hygienic Laboratory and the strain of *Bacillus typhi exanthematici* kindly furnished by Dr. Plotz.

Anaërobic Fluid Media Cultures.

In an endeavor to cultivate the Rocky Mountain spotted fever virus in fluid media under lessened oxygen pressures, the following technique was evolved at the field laboratory, Victor, Mont.:



Articles required:

Ten c. c. homeopathic vials, rubber stoppers to fit.
One-fourth inch glass tubing in 6-inch lengths.
One hand vacuum pump.

A constriction was drawn in the glass tubing, as shown in the accompanying illustration, and a small hole, into which the tubing would fit snugly, was burnt in the rubber stopper. The stoppers with tube inserted and the vials were sterilized separately. After the vials had been filled with 8 c. c. of media and inoculated, the stoppers were driven in tightly and sealed with paraffine, and the glass tube was attached to the hand pump. After from 5 to 30 minutes' exhaustion the glass tube was sealed at the constriction previously made and the culture then placed in the incubator.

While there was no exact measure of the vacuum obtained, or of its duration, it was possible to inhibit completely the growth of aërobic organisms by this method when so desired.

The media used were human serum and guinea pig serum with normal salt solution in different dilutions (1 to 2 and 1 to 3) and ascitic fluid undiluted; a piece of fresh guinea pig kidney was added at the time of inoculation in practically all instances. The material employed in inoculating the media consisted of infected guinea pig blood and tissues, blood from human cases of spotted fever, and infected tick eggs.

The following is a brief summary of the results obtained: Forty-five series of vials were planted. In the beginning so much time was consumed in searching smears made from the cultures for micro-organisms that it was decided to depend entirely upon animal inoculations, followed by immunity tests, in order to determine if possible the presence of the living virus in the cultures.

Ninety-seven guinea pigs were inoculated from these cultures and later tested for immunity by the injection of 0.5 c. c. of known spotted fever virus.

The cultures tested were from two days to one month old, the majority being less than two weeks old. All the guinea pigs injected with cultures less than two weeks old, when later given the immunity test, developed spotted fever.

Three guinea pigs out of ten inoculated with cultures 21 to 25 days old, either showed definite lesions of spotted fever or were immune to the spotted fever virus, as shown below.

Series No.	Culture medium.	Material planted.	Method.	Animal inoculations.	Immunity tests.	Remarks.
C1—June 15, 1915.	8 c. c. dilute human serum + normal g. p. kidney.	5 drops seventh day g. p. blood.	Air exhausted, vial sealed and kept at 37°.	July 7, 5 drops injected into g. p.	G. p. immune to spotted fever virus injected June 30 and again on Aug. 6, 1915.	G. p. showed temperature above 40° C. June 17 to 22, 1915.
D2—June 17, 1915.	.....do.....	7 drops human spotted fever blood.	.....do.....	June 29, 5 drops injected into g. p. D2a. July 9, 5 drops injected into g. p. D2b.	G. p. D2a injected Aug. 28 developed spotted fever. G. p. D2b injected Aug. 13 and found immune.	G. p. D2b showed characteristic lesions of spotted fever following injection of July 9, 1915.
H3—July 15, 1915.	.....do.....	Infected tick eggs crushed.	.....do.....	Aug. 10, 5 drops injected into g. p.	G. p. immune to spotted fever virus injected Aug. 28, 1915.	

Inasmuch as the Rocky Mountain spotted fever virus ordinarily dies within 24 to 48 hours when kept at a temperature of 37°, as the dosage of the cultures injected was much less than the minimum infective dose of fresh virus, and as in the inoculation of several hundred guinea pigs no naturally immune guinea pig has been encountered, it seems reasonable to conclude that a multiplication of the virus occurred in the cultures C1, D2, and H3.

Centrifugation of the Virus.

Ricketts was unable to throw down the virus from guinea pig and monkey serum when diluted with equal parts of salt solution, even after prolonged centrifugation (six hours). Centrifugation with greater dilutions of normal salt solution has been employed by the writer several times successfully for the purpose of freeing the virus from a coccus contamination. In this way, by injecting different layers of the centrifuged material, a layer was found which would produce spotted fever in the inoculated guinea pig, without carrying over the contaminating coccus.

By increasing the dilution to 1 part of serum to 8 or 10 of salt solution it was found that the spotted fever virus could be thrown

down completely by four to six hours' centrifugation, as is shown in the following experiment:

After defibrinating 10 c. c. of spotted fever blood and centrifuging for 15 minutes, 1 c. c. of the serum was pipetted off and diluted with 10 parts of normal salt solution. This was then centrifuged for four and one-half hours at about 2,000 revolutions per minute. Ten c. c. of the supernatant fluid was carefully drawn off and injected into guinea pig S2. This pig showed no reaction whatever, and later developed spotted fever when inoculated with the virus; while guinea pig S1, inoculated with three drops of the sediment, developed spotted fever on the eighth day following inoculation and showed all the characteristic lesions of the disease as seen in the guinea pig. This experiment has been repeated many times with similar results.

Microscopical Examination of Spotted-Fever Blood.

Stimulated by the fact that the virus of Rocky Mountain spotted fever does not pass through a Berkefeld filter (N) under a pressure of 1 atmosphere, various investigators of the disease have spent much time in searching fresh blood smears for the causative organism, but without agreement as to findings.

During the last three years the writer has examined many blood smears prepared and stained by all the well-known methods, from human cases and from the known susceptible animals, particularly guninea pigs and white rats. Frequently there have been found in spotted fever blood, stained by the Giemsa method, extra corpuscular granules, singly and in pairs, staining bright red and highly refractile; also, similar bodies within or in close proximity to the erythrocytes. The intracellular bodies are usually surrounded by a pale halo. The presence of these granules was considered significant, but it has been impossible to differentiate them with certainty from the granules sometimes found in normal blood.

By dilution and centrifugation a method for concentrating and distinguishing these bodies appears to have been found, the best results having been obtained in the following manner: Ten c. c. of infected blood is withdrawn by heart puncture, defibrinated, and immediately centrifuged for 15 minutes. One c. c. of the surface serum is then pipetted off and diluted with 10 c. c. of normal salt solution in an ordinary centrifuge tube. One c. c. of the remaining serum, containing some of the upper layer of red cells, is treated in the same way. These fluids are then centrifuged for 6 hours, the supernatant fluid is carefully poured off, and smears are made from the drop of sediment remaining and stained over night with dilute Giemsa stain.

The serum smears show many bright red granular bodies, singly and in pairs, highly refractile, accompanied by larger light-blue bodies, and all surrounded by a pale-blue matrix, the whole mass

being rather indistinct but not encountered in the controls. The red blood cells appear to take the stain normally, but in many of them are found round or slightly elongated red chromatin bodies partially surrounded by or in close approximation to a somewhat larger deep-blue staining body. Some of the chromatin bodies approach 1 μ in diameter, but the majority are smaller and in these the protoplasm is elongated, extending well beyond the chromatin body at both ends.

Some of the bodies are found clearly without the cells and in the largest of these the red chromatin body is centrally located and surrounded entirely by the deep-blue staining protoplasm, the whole being crescentic in shape.

This method of preparing and staining blood smears has been repeated many times with proper controls of normal guinea-pig blood and with blood from pigs sick with diseases other than spotted fever, with the result that the bodies above described have never been found except in spotted fever blood.

There appears to be some resemblance between these bodies found in spotted fever guinea pig blood and those described by Seidelin as having been found by him in yellow fever blood; and in view of the criticism of Seidelin's work made by Wenyon and Low, who claim to have found similar bodies in normal guinea pig blood, one naturally hesitates to draw any definite conclusions from the finding here reported.

From the fact that these bodies, on account of their morphological and tinctorial characteristics, may be regarded as probably of protozoan nature, and because they have thus far been found only in blood from animals infected with spotted fever, it is felt that the publication of their description at this time is justified, in order that other workers may be on the lookout for them, and that their relationship to Rocky Mountain spotted fever may be fully established.

The writer is indebted to Surg. A. M. Stimson and Asst. Surg. R. R. Spencer for assistance in carrying on the above-described investigations.

PLAGUE-PREVENTION WORK.

CALIFORNIA.

The following reports of plague-prevention work in California were received from Surg. Boggess, of the United States Public Health Service, in charge of the work.